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Immobilized Phosphorylase for Synthesis of Polysaccharides from Glucose

Continuous processes for the enzymatic production of carbohydrates from glucose require that enzymes be immobilized or rendered insoluble so that they can be readily separated from process streams. A number of enzymes have been successfully immobilized, but not of the type which form high molecular weight polysaccharides.

One of the key reactants in a system leading to carbohydrate synthesis is a phosphorylase which catalyzes the reversible formation or degradation of polysaccharide (or starch-like) material; in a continuous process, the enzyme is preferably immobilized by attachment to a water-insoluble support. For a systematic program of research, colloidal silica-cellulose and a porous "alkylamine glass" were selected as the insoluble substrates for immobilization of phosphorylase. Two different methods were developed for immobilizing phosphorylase on the substrates, and it was found that the products have widely different binding capacities as well as physical properties. It was also found that the amount of enzyme that can be bound to a unit weight of porous glass is less than can be bound to a silica-cellulose support; of greater interest, however, is the finding that the physical properties of the alkylamine glass product are superior for the flow-through operations involved in a practical process for forming polysaccharides.

Fumed silica was treated with polyethyleneimine to form an intermediate layer which could absorb purified potato phosphorylase; after treatment with the enzyme, glutaraldehyde was crosslinked to the product, and the composite was treated with an

active-ester cellulose to form an immobilized phosphorylase-silica-cellulose complex.

Alkylamine glass, with an essential surface character consisting of $(H_2N-R-Si-O-)$ groupings, was treated with glutaraldehyde until all active groupings had been derivatized. The glutaraldehyde-glass product was washed with water and then treated with a solution of phosphorylase to form an immobilized complex.

The activity of each of the immobilized phosphorylase products was determined in buffered solutions of glucose-1-phosphate containing a specially fractionated amylopectin (primer). The primer is a polysaccharide onto which the enzyme adds successive glucose residues; the concentration and size of the primer controls the molecular weight of the polysaccharide formed by the immobilized phosphorylase. The silica-cellulose-phosphorylase complex is about five times more active than the corresponding glass complex.

The phosphorylase-glass complex was made into a column and then used to catalyze the formation of polysaccharide from glucose on a continuous basis. The column was maintained at $45^\circ C$ to enhance the reaction rate and solubility of the polysaccharide product. When the enzyme column was operated under conditions which produced a product too high in molecular weight, a polysaccharide coating formed on the glass surface and decreased the apparent activity of the column. This coating could be removed by a starch-hydrolyzing enzyme (glucoamylase); the treatment restored the column efficiency nearly to the original level.

(continued overleaf)

Notes:

1. The alkylamine glass can be treated with 2-amino-4,6-dichloro-s-triazine followed by phosphorylase in borate buffer to give another form of an immobilized phosphorylase-glass complex, but it is not as active as the complex described above.
2. Requests for further information may be directed to:

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No patent action is contemplated by NASA.

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